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CHEMOTHERAPEUTIC STUDIES WITH ETHYLHYDROCUPREIN AND MERCUROPHEN IN EXPERIMENTAL PNEUMOCOCCUS MENINGITIS IN RABBITS

JOHN A. KOLMER AND GORO IDZUMI

From the McManes Laboratory of Experimental Pathology of the University of Pennsylvania, Philadelphia

Pneumococcus meningitis, whether originating as a local infection of the cerebral meninges by extension from the ear and accessory nasal sinuses, during pneumonia, or developing as a spontaneous infection without a discoverable primary lesion, is a highly fatal disease; in the experience of one of us with fourteen cases of this infection, death resulted in all. Fortunately the disease is not common and does not occur in epidemics; reliable statistics are not available, but an analysis of the available literature shows that the mortality is over 90%.

Serum treatment of this infection either with polyvalent serums or the monovalent serum for type 1 infections, has failed materially to influence the disease; the studies of Lamar[1] with experimental pneumococcus meningitis in monkeys, have shown that the serum alone has but slight curative value, although encouraging and frequently brilliant results were observed with mixtures of serum, sodium oleate and boric acid, the sodium oleate rendering the organisms more vulnerable to the serum. These results were observed only when specific serum was employed, that is, the serum corresponding to the type of pneumococcus producing the disease; the antiserum for type 1 was ineffective for type 2 infections even in the presence of sodium oleate.

Systematic studies in the chemotherapy of pneumococcus meningitis do not appear to have been made; owing to the comparative ease with which the disease may be produced experimentally, providing highly virulent cultures are available, it would appear a particularly interesting field for experimental study under conditions closely resembling a disease occurring among persons. The fact that the subarachnoid space is practically a closed one with circulation of the cerebrospinal

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[1] Jour. Exper. Med., 1912, 16, p. 581.

fluid resulting in a diffusion of substances introduced in the lumbar regions until such time as inflammatory changes interfere, renders experiments in meningitis particularly serviceable in chemotherapeutic studies in bacterial infections.

PURPOSES OF INVESTIGATION

In the chemotherapy of pneumococcus infections various compounds of quinin, and particularly the optochin of Morgenroth and Levy,[2] have given the most encouraging results. As a result of the work of these investigators in addition to that of Moore[3] and Cohen, Kolmer and Heist[4] in this country, showing the high pneumococcidal activity of ethylhydrocuprein hydrochlorid in test tube experiments and some protective and curative activity in experimental pneumococcus infections in mice,[5] we decided to study the probable influence of this substance alone and in combination with antipneumococcus serum and sodium oleate on pneumococcus meningitis experimentally produced in rabbits. Additional experiments were conducted with some of the ordinary soluble salts of quinin, notably the hydrobromid, and also with sodium oxymercury ortho-nitro-phenolate prepared by Schamberg, Kolmer and Raiziss,[6] a mercurial designated "mercurophen" and possessing certain superior properties as high germicidal activity for pathogenic cocci notably staphylococci and pneumococci, lesser toxicity as compared with other mercurials on the basis of content of mercury, and remarkable freedom from precipitating influence on serum proteins.

Our experiments were conducted by first determining the toxicity of these various substances by subthecal injection in rabbits followed by a study of their influence in varying dosage on the course of infections produced by the subthecal injection of virulent pneumococci; the object of this paper is to record briefly the results of these experiments with ethylhydrocuprein hydrochlorid and mercurophen inasmuch as the former particularly yielded encouraging results and may prove of some aid in the treatment of pneumococcus meningitis during the early stages of this highly mortal disease.

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[2] Berl. klin. Wchnschr., 1911, 48, pp. 1561, 1650, 1779, 1983.

[3] Jour. Exper. Med., 1915, 22, p. 269.

[4] Jour. Infect. Dis., 1917, 20, p. 272.

[5] Moore, H. F.: Jour. Exper. Med., 1915, 22, p. 551 Cohen, S. S.: Kolmer, J. A., and Heist, G. D.: Jour. Infect. Dis., 1917, 20, p. 313.

[6] Jour. Infect. Dis., 1919, 24, p. 547.

EXPERIMENTAL PNEUMOCOCCUS MENINGITIS

The lesions were produced in rabbits by the subthecal injection in the lumbar region of virulent pneumococci suspended in physiologic salt solutions; as to be described,[7] a definite train of lesions and symptoms follow in the nature of an acute suppurative leptomeningitis involving the spinal cord and base of the brain accompanied by cloudy spinal fluid, fever, leukocytosis, hyperesthesia, opisthotonus and convulsions.

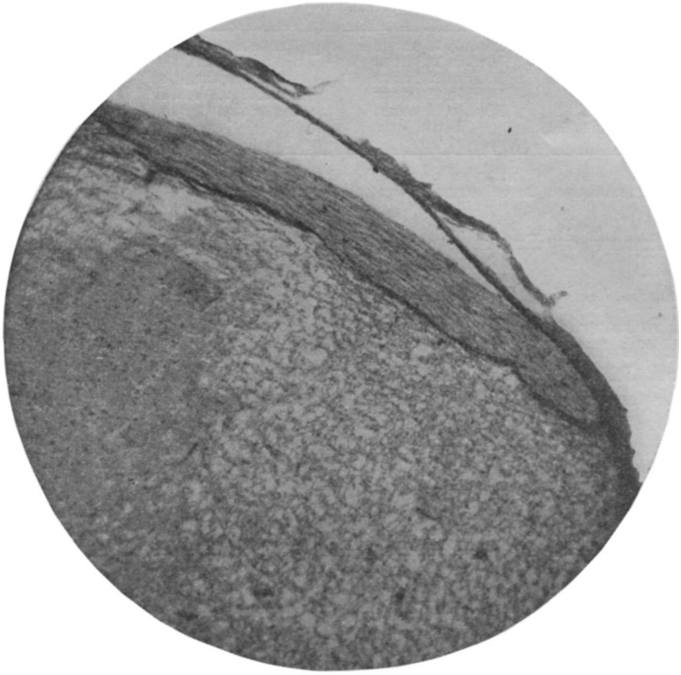

Fig. 1.—Section of spinal cord in dorsal region of a rabbit succumbing 36 hours after the subthecal injection in the lumbar region of 0.5 c c of 1: 100 ethylhydrocuprein per kilogram of weight; shows slight hyperemia and leukocytic infiltration of the meninges with involvement of the adjacent cord substance; × 70.

Types 1 and 2 strains of pneumococci were used; the latter were freshly cultivated from the spinal fluid of a fatal case of meningitis. The type 1 strain proved fatal for white mice of about 20 gm. in weight in 48 hours in a dose of 0.001 c c of 18 to 24 hour broth culture; the type 2 strain was more virulent inasmuch as it regularly killed mice in 48 hours in a dose of 0.00001 c c of 24-hour broth cultures. According to body weight, rabbits required about ten times larger doses injected subthecally to produce a fatal leptomeningitis accompanied by pneumococcus bacteremia; for example, rabbits weighing about 2,000 gm. required 0.01 c c of type 2 to produce a fatal meningitis in about 48 hours, which calculated on the base of body weight, is ten times greater than the minimum lethal dose for mice by intraperitoneal injection (table 1). Smaller doses of these cultures produced milder forms of meningitis and prolonged life for one or more days.

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[7] Idzumi, G.: Experimental Pneumococcus Meningitis in Rabbits and Dogs. This journal.

TABLE 1

VIRULENCE OF TYPE 2 PNEUMOCOCCUS FOR RABBITS BY INTRASPINAL INJECTION

Weight in Grams	Dose in c c	Results	Necropsy	Cultures at Necropsy	
				Heart	Spinal Fluid
2,300	1.0	Died in 12 hours	Acute meningitis	+*	+
1,800	0.5	Died in 12 hours	Acute meningitis	+	+
2,000	0.1	Died in 26 hours	Acute meningitis	+	+
2,100	0.01	Died in 38 hours	....	+	+
1,800	0.001	Died in 72 hours	Acute meningitis	+	+
1,700	0.0001	Lived			

Virulent for mice by intraperitoneal injection 0.00001 c c in 48 hours.
* + = pneumococci in cultures; — = sterile.

TOXICITY OF ETHYLHYDROCUPREIN HYDROCHLORID FOR RABBITS BY SUBTHECAL INJECTIONS

According to Moore,[5] solutions of ethylhydrocuprein hydrochlorid slowly injected intravenously into rabbits proved immediately fatal in doses of about 0.06 gm. per kilo of body weight, the tolerated dose being 0.02 to 0.05 gm. per kilo; Cohen, Kolmer and Heist[5] found the immediate lethal dose about 0.06 gm. per kilo and the highest tolerated dose about 0.02 to 0.04 gm. per kilo of body weight in a period of 10 days. As is well known, the drug may produce a toxic amblyopia in persons, and its therapeutic usefulness has been thereby reduced.

TABLE 2

TOXICITY OF ETHYLHYDROCUPREIN HYDROCHLORID BY INTRASPINAL INJECTION

Weight in Grams	Dose per kilo	Equivalent in Grams	Results	Necropsy
1,700	0.5 c c of 1:50	0.01	Died in 12 hours	Acute congestion; meningitis
2,300	0.5 c c of 1:100	0.005	Died in 36 hours	Acute meningitis
2,400	0.5 c c of 1:500	0.001	Lived indefinitely	
1,800	0.5 c c of 1:500	0.001	Killed in 48 hours	Mild meningitis
2,000	1.0 c c of 1:500	0.002	Killed in 96 hours	Mild meningitis
2,000	0.5 c c of 1:1,000	0.0005	Lived indefinitely	
1,800	0.5 c c of 1:1,000	0.0005	Killed in 48 hours	No meningitis
2,000	1.0 c c of 1:1,000	0.001	Killed in 96 hours	No meningitis
2,500	0.5 c c of 1:2,000	0.00025	Lived indefinitely	
1,700	0.5 c c of 1:5,000	0.0001	Lived indefinitely	

Solutions of the drug in salt solution injected subthecally in rabbits have proved fatal in doses of 0.03 to 0.05 gm. per kilo of body weight in 48 hours as shown in table 2. These amounts dissolved in 0.5 c c salt solutions per kilo of weight produce paralysis of the hind legs in the majority of animals and an acute meningitis characterized by marked hyperemia and slight leukocytic infiltration of the meninges, especially in the lumbar region near the site of injection, but also extending to the base of the brain. Smaller doses as 0.001 gm. in 0.5 c c of salt solution per kilo of body weight usually do not produce symptoms, but may produce slight congestion of the meninges at the site of the injection; 0.0005 gm., equivalent to 0.5 c c of a 1:1,000 dilution per kilo, did not produce any symptoms or discernible changes in the meninges and has been generally employed by us in the therapeutic experiments. This dose corresponds to 30 c c of a 1:1,000 dilution per 60 kilo of weight and represents a safe dose of ethylhydrocuprein hydrochlorid for adult persons by subthecal administration.

TOXICITY OF MERCUROPHEN FOR RABBITS BY SUBTHECAL INJECTION

Schamberg, Kolmer, Raiziss and Trist have found the tolerated dose of mercurophen for white rats by intravenous injection to be 0.006 to 0.008 gm. per kilo of weight over a period of 10 days; by subthecal injection in rabbits we have found the lethal dose of mercurophen about 0.002 to 0.004 gm. per kilo of body weight in about 48 hours (table 3); these doses produce paralysis of the hind legs and a severe meningitis characterized by intense hyperemia and hemorrhagic extravasation in the meninges, particularly in the neighborhood of the site of injection in the lumbar regions. In dose of 0.0005 gm. per kilo of weight, equivalent to 1 c c of a 1:2,000 dilution, mercurophen does not produce paralysis or prove fatal for normal rabbits but may produce slight hyperemia of the meninges in the immediate site of injection; when injected subthecally in rabbits with pneumococcus meningitis the toxic effects are more marked and may hasten the death of the animal as compared with untreated control animals.

TABLE 3

TOXICITY OF MERCUROPHEN FOR RABBITS BY INTRASPINAL INJECTION

Weight in Grams	Dose per Kilo	Equivalent in Grams	Results	Necropsy
1,700	1 c c of 1:500	0.002	Paralysis; died in 58 hours	Acute hemorrhagic meningitis
1,900	1 c c of 1:1,000	0.001	Slight paralysis; lived*	Mild meningitis at site of injection
2,200	1 c c of 1:2,000	0.0005	No paralysis; lived*	No meningitis
2,100	1 c c of 1:5,000	0.0002	No paralysis; lived*	No irritation of meninges

* Killed after 96 hours.

Therefore, both ethylhydrocuprein and mercurophen are equally or probably slightly more toxic when injected subthecally than when introduced directly into the venous blood; probably this is due in part to the local injury consequent to lumbar puncture and more especially to the direct effect of these substances on the delicate nervous structures with which they are brought into immediate contact.

THE PNEUMOCOCCIDAL ACTIVITY OF ETHYLHYDROCUPREIN HYDROCHLORID AND MERCUROPHEN IN THE SPINAL FLUID OF ACUTE MENINGITIS

While both ethylhydrocuprein hydrochlorid and mercurophen possess extremely high germicidal values for pneumococci in a broth or salt solution, this activity is materially reduced in the presence of large amounts of serum proteins and it was necessary to determine their pneumococcidal activity in the spinal fluids of acute meningitis before undertaking experiments on their influence in experimental pneumococcus meningitis.

For this purpose turbid spinal fluids containing many thousands of pus cells per cubic millimeter and yielding strongly positive Pandy and Noguchi reactions for proteins were used. In one of these fluids within a few hours after removal from a person with meningitis, type 2 pneumococci identified by serologic methods were present in large numbers and were mostly extracellular; in other experiments spinal fluid containing type 1 pneumococci (extracellular) was employed.

In the tests varying amounts of spinal fluid were mixed with a solution of the drugs in sterile salt solution and the mixtures kept on a water bath at 38 C.; at varying intervals from 2 to 4 loopfuls of each mixture were subcultured in tubes of glucose blood broth and observed over a period of 72 hours to determine germicidal activity. The results of several experiments shown in tables 4, 5 and 6 give the amounts of spinal fluid and drug employed and the final dilutions.

TABLE 4

GERMICIDAL ACTIVITY OF ETHYLHYDROCUPREIN AND QUININ HYDROBROMID FOR TYPE 2 PNEUMOCOCCI IN SPINAL FLUID

Drug 1 c c	Spinal Fluid	'Final Dilution	Results of Subcultures after Exposure for Minutes at 28 C.					
			5	15	30	45	60	90
Ethylhydrocuprein 1:1,000.........	1 c c	1:2,000	+*	+	—	—	—	—
Ethylhydrocuprein 1:2,000.........	1 c c	1:4,000	+	+	+	+	—	—
Ethylhydrocuprein 1:5,000.........	1 c c	1:10,000	+	+	+	+	+	—
Ethylhydrocuprein 1:25,000........	1 c c	1:50,000	+	+	+	+	+	+
Quinin hydro. 1:100................	1 c c	1:200	+	+	+	+	—	—
Quinin hydro. 1:200................	1 c c	1:400	+	+	+	+	+	+
Salt solution......................	1 c c	....	+	+	+	+	+	+

* + = pneumococci; — = sterile.

TABLE 5

GERMICIDAL ACTIVITY OF MERCUROPHEN FOR TYPE 2 PNEUMOCOCCI IN SPINAL FLUID OF MENINGITIS

Spinal Fluid, c c	Mercurophen, 0.1 c c	Final Dilution	Results of Subcultures after Exposure for Minutes at 38 C.					
			5	15	30	45	60	120
0.3	1:250	1:1,000	—*	—	—	—	—	—
0.3	1:500	1:2,000	—	—	—	—	—	—
0.2	1:1,000	1:3,000	+	—	—	—	—	—
0.3	1:1,000	1:4,000	+	+	+	+	—	—
0.5	1:1,000	1:6,000	+	+	+	+	+	+
0.7	1:1,000	1:8,000	+	+	+	+	+	+
1.0	None	....	+	+	+	+	+	+

* — = sterile; + = pneumococci.

TABLE 6

GERMICIDAL ACTIVITY OF MERCUROPHEN FOR TYPE 1 PNEUMOCOCCI IN SPINAL FLUID OF MENINGITIS

Spinal Fluid, c c	Mercurophen, 0.1 c c	Final Dilution	Results of Subcultures after Exposure for Minutes at 38 C.					
			5	15	30	45	60	120
0.3	1:250	1:1,000	—*	—	—	—	—	—
0.3	1:500	1:2,000	—	—	—	—	—	—
0.2	1:1,000	1:3,000	—	—	—	—	—	—
0.3	1:1,000	1:4,000	+	+	—	—	—	—
0.5	1:1,000	1:6,000	+	+	+	+	+	+

* — = sterile; + = pneumococci.

As shown in table 4 ethylhydrocuprein hydrochlorid proved germicidal for type 2 pneumococci in dilutions of 1:2,000 in 30 minutes; in 1:4,000 in one hour and 1:10,000 in 1½ hours; quinin hydrobromid was much less active proving germicidal in but 1:200 in an hour. Inasmuch as ethylhydrocuprein could be used in rabbits in a dose of 0.5 c c of 1:1,000 dilution per kilo of weight, and assuming that there are about from 2 to 3 c c of spinal fluid per kilo of weight equivalent to from 120 to 180 c c per adult of about 125 pounds, the final dilution of the drug in the spinal fluid of normal rabbits was about 1:6,000 providing diffusion occurred. Experiments with dye stuffs have shown that 0.5 c c of 1% solutions injected subthecally in the lumbar region of rabbits diffuse along the entire cord and base of the brain within from 3 to 6 hours, but may not be found in the ventricles; accordingly the final dilution of drug in the subarachnoid space may not be as high as stated, and in view of the results observed with germicidal tests in vitro with purulent spinal fluids it was reasonable to expect that sufficient amounts of ethylhydrocuprein hydrochlorid could be injected subthecally to influence the course of a pneumococcus meningitis even though the amount of spinal fluid was increased by the inflammatory processes. In view of the much lower pneumococcidal activity of the ordinary soluble salts of quinin in purulent spinal fluids and since their toxicity is close to that of ethylhydrocuprein,[5] we did not regard it possible to influence the course of experimental meningitis and experiments subsequently showed the truth of this assumption.

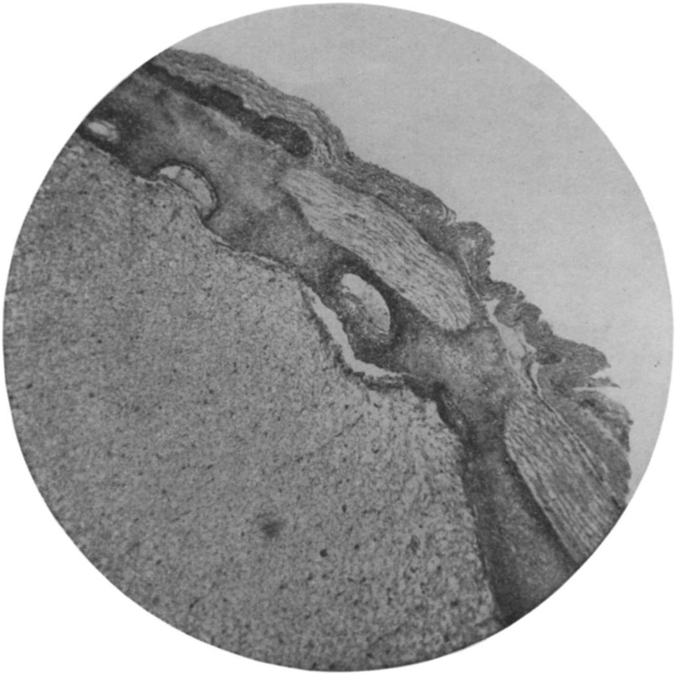

Fig. 2.—Section of spinal cord of rabbit in dorsal region succumbing 48 hours after the subthecal injection in the lumbar region of 0.5 c c of 1:500 mercurophen per kilogram of weight; shows marked hemorrhagic meningitis; × 70.

Similar experiments with mercurophen have shown a well defined pneumococcidal activity; as shown in table 5, this substance proved completely germicidal in 5 minutes in a final dilution of 1:2,000 and in 60 minutes in a final dilution of 1:4,000 employing the same purulent spinal fluid used in the tests with ethylhydrocuprein hydrochlorid. Similar results were observed with type 1 pneumococci (table 6) and indicate that while mercurophen acts somewhat more quickly and is more germicidal than ethylhydrocuprein hydrochlorid in short exposures, the latter excels somewhat in the larger exposures. Owing, however, to the much higher toxicity of mercurophen, we did not believe that a sufficient amount could be injected subthecally in one dose to impart to the spinal fluid well defined germicidal activity. Subsequent experiments have shown this to be true.

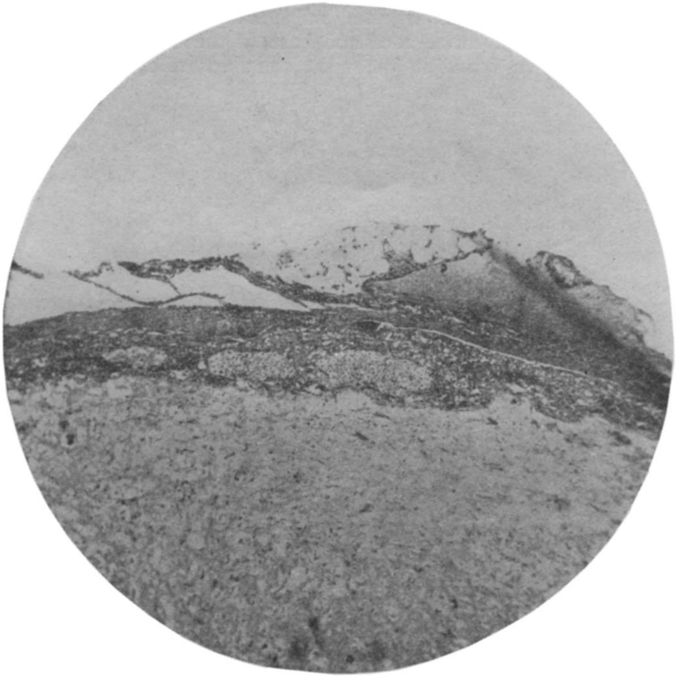

Fig. 3.—Section of spinal cord of rabbit in dorsal region succumbing four days after the subthecal injection of type 1 pneumococci in the lumbar region; shows purulent leptomeningitis; × 70.

THE INFLUENCE OF ETHYLHYDROCUPREIN HYDROCHLORID ON EXPERIMENTAL PNEUMOCOCCUS MENINGITIS

With these preliminary experiments finished, the study was continued by producing severe leptomeningitis in rabbits by the subthecal injection in the lumbar region of type 1 or type 2 pneumococci suspended in warm salt solution. At varying intervals the drug was injected subthecally about two interspaces higher in the lumbar region and the animals carefully observed over a period of several days. Temperature observations and leukocyte counts were made at regular intervals and the influence of ethylhydrocuprein determined by these observations in addition to the clinical condition of each animal and the duration of its life as compared with numerous controls. Owing to the small size of the animals, it was not always possible to secure spinal fluid for examination; a drop or two was the largest amount that could be secured suitable for films and permitting observations on the nature of the exudate in the spinal fluid and the degree of phagocytosis. The clinical symptoms, duration of life and results of complete postmortem examinations, including a histologic examination of the cord and brain of each animal, were the chief criteria for judging the results which are presented in the form of tables and omitting detailed descriptions of individual animals for the purpose of brevity.

Table 7 shows the results of one experiment employing type 1 pneumococci; the control died on the fourth day with well defined leptomeningitis. A large dose of ethylhydrocuprein as 0.5 c c of a 1: 100 dilution per kilo of body weight produced paralysis in a control and hastened the death of an infected animal; smaller doses of the drug, however, prolonged the lives of several animals over an indefinite period when injected within 4 hours of the pneumococci. When the infection had been given a 24 or 48-hour start, the influence of the drug in prolonging life was not evident.

TABLE 7

INFLUENCE OF ETHYLHYDROCUPREIN HYDROCHLORID ON TYPE 1 MENINGITIS OF RABBITS

Weight in Grams	Dose of Drug per Kilo	Time of Injection of Drug in Relation to Infection	Result in Days							Lesions
			1	2	3	4	5	6	7	
1,600	0.5 c c of 1:100	At once	T	D	...	...	...	...	...	Acute congestion
1,500	0.5 c c of 1:500	4 hours	T	T	T	R	R	R	R	Paralysis
1,900	0.5 c c of 1:1,000	4 hours	T	T	R	R	R	R	R	
2,000	0.5 c c of 1:5,000	4 hours	T	T	T	T	T	R	R	
2,000	0.5 c c of 1:500	24 hours	T	T	T	T	D	...	...	Meningitis
2,100	0.5 c c of 1:500	48 hours	T	T	T	D	...	...	...	Meningitis
1,700	0.5 c c of 1:100	Control	T	T	R	R	R	R	R	Paralysis; recovered
1,800	1.0 c c salt sol.	Control	R	R	R	R	R	R	R	No paralysis
2,000	Control (culture)	....	T	T	T	D	...	...	...	Meningitis

Culture used in dose of 0.5 c c.
T = toxic; R = recovered; D = died.

These results are illustrative of those obtained in similar experiments employing this culture which possessed a moderate degree of virulence; even as small doses of drug as 0.5 c c of 1: 5,000 dilution per kilo of body weight exerted a well defined protective value, providing the administration was within a few hours of the injection of cocci. These results were probably due to the actual destruction of the pneumococci while the organisms were confined to the subarachnoid space of the spinal cord; when well defined and widespread inflammatory changes became apparent with an increased volume of spinal fluid containing numerous pus cells, flakes of fibrin and great numbers of pneumococci, the curative influence of a single dose of ethylhydrocuprein was greatly reduced.

With the more virulent type 2 strain the curative value of a single dose of ethylhydrocuprein hydrochlorid was feeble, as shown in table 8; this culture in the dose employed, produced a particularly severe form of leptomeningitis and rendered the experiments unfavorable for the discovery of minor degrees of curative activity of the drugs studied. The controls usually died in about 48 hours with suppurative leptomeningitis; a single dose of ethylhydrocuprein would prolong the life for 1 or 2 days providing the drug was administered within 4 to 6 hours after the cocci.

TABLE 8

INFLUENCE OF ETHYLHYDROCUPREIN HYDROCHLORID ON TYPE 2 MENINGITIS OF RABBITS

Weight in Grams	Dose of Drug per Kilo	Time of Injection of Drug	Results in Days			Necropsy
			1	2	3	
1,700	1 c c of 1:1,000	2 hours after cocci	T†	T	D	Acute meningitis
1,500	1 c c of 1:1,000	6 hours after cocci	T	T	D	Acute meningitis
2,000	1 c c of 1:1,000	24 hours after cocci	T	D	..	Acute meningitis
2.000	0.5 c c of 1:1,000‡	2 hours after cocci	T	D	..	Acute meningitis
1,500	Control	....	T	D	..	Acute meningitis

* Culture used in dose of 0.005 c c of 24-hour culture intraspinally.
† T = toxic; D = died.
‡ Administered intravenously.

In other experiments consisting in the repeated injection of smaller doses of pneumococci followed 6 hours later by a subthecal injection of ethylhydrocuprein, in order to avoid an overwhelming infection and to similate more closely the method of procedure in the treatment of human infections, ethylhydrocuprein hydrochlorid showed a well defined curative or protective activity inasmuch as the treated animals generally survived for longer periods than the controls. As shown in tables 9 and 10, the repeated injection of the drug in dose of 0.5 c c of 1:1,000 solution per kilo of weight had no discernible injurious effect; several injections of cocci alone generally produced a fatal meningitis whereas in animals receiving the same doses of culture followed after an interval by a subthecal injection of the drug life was prolonged, in one instance for 12 days beyond the life of the control.

TABLE 9

INFLUENCE OF ETHYLHYDROCUPREIN HYDROCHLORID ON TYPE 2 MENINGITIS OF RABBITS

Weight in Grams	Culture c c*	Drug, Dose per Kilo†	Results
1,800	0.0001	None (control)	Died on 6th day
2,000	0.0001	0.5 c c of 1:1,000	Died on 12th day
2,000	0.0001	0.5 c c of 1:1,000	Died on 18th day
2,000	None	0.5 c c of 1:1,000	Lived indefinitely

* Culture 0.0001 c c given intraspinally on 1st, 3rd, 5th, 7th, 9th and 11th days.
† Administered intraspinally 6 hours after each injection of cocci.

TABLE 10.

THE INFLUENCE OF DAILY INJECTIONS OF ETHYLHYDROCUPREIN HYDROCHLORID ON TYPE 2 MENINGITIS OF RABBITS

Weight in Grams	Culture c c	Drug, Dose per Kilo*	Results
2,000	0.0001	0.5 c c of 1:1,000	Died on 6th day
2,000	0.0001	None	Died on 3rd day

* Daily subthecal injections.

Experiments consisting in the intravenous injection of ethylhydrocuprein had no appreciable effect on the course of experimental meningitis.

Since the investigations of Kolmer, Cohen and Steinfield[8] have shown that the presence of ethylhydrocuprein hydrochlorid in leukocytic mixtures increases the phagocytosis of pneumococci, certain experiments were specially planned employing large rabbits for the purpose of conducting repeated examinations of the spinal fluids during the course of meningitis produced by the type 2 strain with and without treatment with ethylhydrocuprein. As previously stated, only sufficient fluid could be obtained during life for films; total cell counts were generally inaccurate owing to the presence of traces of blood.

Within a few hours after the injection of pneumococci many polymorphonuclear leukocytes were to be found in the spinal fluid; at the end of 24 hours these numbers had increased so rapidly along with an enormous proliferation of the organisms as to render the fluid distinctly turbid. Within 48 hours the fluid was rich in fibrin and particles of plastic exudate were to be found attached to the meninges, cord and base of the brain. The pneumococci were invariably extracellular; only occasionally would a pair or two be found within a polymorphonuclear leukocyte.

Films of fluid removed at varying intervals after the injection of ethylhydrocuprein frequently showed numerous phagocytes and at times swollen diplococci retaining the Gram stain poorly or not at all; evidence of the influence of ethylhydrocuprein on the organisms and the phenomenon of phagocytosis were especially marked when spinal fluid was secured within 4 to 6 hours after the injection of the drug.

INFLUENCE OF ANTIPNEUMOCOCCUS SERUM ALONE AND IN COMBINATION WITH ETHYLHYDROCUPREIN HYDROCHLORID ON EXPERIMENTAL MENINGITIS

Moore[9] has shown that mixtures of antipneumococcus serum type 2 and optochin (base) injected subcutaneously are more protective for mice infected by intraperitoneal injections of virulent pneumococci than either substance alone and beyond what could be expected as the summation of the protective value of each; Kolmer and Steinfield[10] have likewise found that ethylhydrocuprein injected subcutaneously usually increased the protective value of antipneumococcus serum for type 1 infections of mice.

Accordingly, we thought it of interest and value to study the influence of mixtures of antipneumococcus serum and ethylhydrocuprein on type 1 meningitis inasmuch as type 1 infections are occasionally encountered among persons suffering with pneumococcus meningitis.

The serum employed was not as good as it should have been but was the best obtainable at the time these experiments were conducted; this serum agglutinated the type 1 strain in final dilution of 1:32 and in dose of 0.2 c c protected mice against about 10 minimal lethal doses of culture, both tests being conducted after the Rockefeller Institute methods.

Experiments consisting in the treatment of meningitis with the serum alone administered subthecally in single dose of 1 c c per kilo of weight corresponding to 60 c c for a person weighing about 125 pounds and at intervals varying from 2 to 72 hours after the injection of the pneumococci, have generally yielded negative results as shown in one experiment in table 11; in other experiments some slight influence of the serum was apparent on the basis of prolongation of the lives of the experimental animals for 1 to 3 days beyond the duration of the lives of the controls, as shown in an experiment in table 12.

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[8] Jour. Infect. Dis., 1917, 20, p. 334.

[9] Jour. Exper. Med., 1915, 22, p. 389.

[10] Jour. Infect. Dis., 1918, 22, p. 492.

TABLE 11

THE INFLUENCE OF ANTIPNEUMOCOCCUS SERUM ON TYPE 1 MENINGITIS OF RABBITS

Weight in Grams	Serum, Dose per Kilo	Results
1,800	1 c c 2 hours after cocci	Lived 4 days; acute meningitis
2,000	1 c c 24 hours after cocci	Lived 5 days; acute meningitis
2,000	1 c c 48 hours after cocci	Lived 4 days; acute meningitis
1,800	1 c c 72 hours after cocci	Lived 4 days; acute meningitis
2,000†	2 c c 24 and 48 hours after cocci	Lived 9 days; acute meningitis
2,000	None (control)	Lived 5 days; acute meningitis

* Minimum lethal dose for mice 0.001 c c in 60 hours; rabbits injected with 0.5 c c.

† Serum administered intravenously.

TABLE 12

INFLUENCE OF ETHYLHYDROCUPREIN AND ANTIPNEUMOCOCCUS SERUM ALONE, AND IN MIXTURE ON TYPE 1 MENINGITIS OF RABBITS

Weight in Grams	Injection	Time of Injection of Serum or Drug or Mixture after Infection	Days							Necropsy
			1	2	3	4	5	6	7	
1,900	Culture alone* (control)	....	T†	T	D	...	...	...	...	Meningitis
2,500	Serum alone‡	24 hours	T	T	T	D	...	...	...	Meningitis
1,900	Serum alone	48 hours	T	T	T	D	...	...	...	Meningitis
1,900	Serum alone	24 and 48 hours	T	T	T	T	D	...	...	Meningitis
2,000	Ethylhydrocuprein alone§	24 hours	T	T	T	T	D	...	...	Meningitis
2,000	Ethylhydrocuprein alone	48 hours	T	T	T	D	...	...	...	Meningitis
2,300	Ethylhydrocuprein alone	24 and 48 hours	T	T	T	T	D	...	...	Meningitis
2,100	Mixture¶	24 hours	T	T	T	T	D	...	...	Meningitis
1,900	Mixture	48 hours	T	T	T	T	T	T	D	Meningitis
2,100	Mixture	24 and 48 hours	T	T	T	D	...	...	...	Meningitis

* Minimum lethal dose for mice 0.001 c c in 60 hours; rabbits infected with 0.5 c c subthecal injection.

† T = toxic; D = died.

‡ Serum alone injected subthecally in dose of 1 c c per kilogram of weight.

§ Ethylhydrocuprein hydrochlorid alone injected subthecally in dose of 0.5 c c of 1:1,000 dilution per kilo of weight.

¶ Serum 0.5 c c and ethylhydrocuprein 0.5 c c of 1:1,000 dilution per kilo of weight.

On the other hand, the repeated intravenous injection of the serum occasionally resulted in prolonging the lives of our rabbits, in one instance for 4 days as shown in table 11; we have not followed out this plan of treatment with a sufficiently large series of animals to determine more definitely the influence of intravenous injection of serum, but it is probable that these effects are due to the influence of the serum on the pneumococcus bacteremia, which invariably accompanies experimental pneumococcus meningitis in rabbits.

Mixtures of serum and ethylhydrocuprein hydrochlorid in preparation of 0.5 c c serum and 0.5 c c of 1: 1,000 solution of drug per kilo of body weight, yielded irregular results in the treatment of experimental meningitis difficult to evaluate, but as a general rule these results were not superior to those observed with the drug alone, as shown in an experiment in table 12.

THE INFLUENCE OF ETHYLHYDROCUPREIN HYDROCHLORID, SODIUM OLEATE AND BORIC ACID ON EXPERIMENTAL PNEUMOCOCCUS MENINGITIS

Lamar having shown that sodium oleate in 0.5 to 1% solution is destructive for pneumococci and that weaker solutions as 0.1 to 0.001% lessen the virulence of these organisms and increase their susceptibility to the destructive action of antipneumococcus serum, boric acid overcoming the inhibitory influence of the protein constituents, we have included a study of these substances alone and in combination with ethylhydrocuprein, on the pneumococcus in infected mice and in rabbits with pneumococcus meningitis, to determine whether sodium oleate increases the susceptibility of virulent pneumococci to the destructive activity of ethylhydrocuprein.

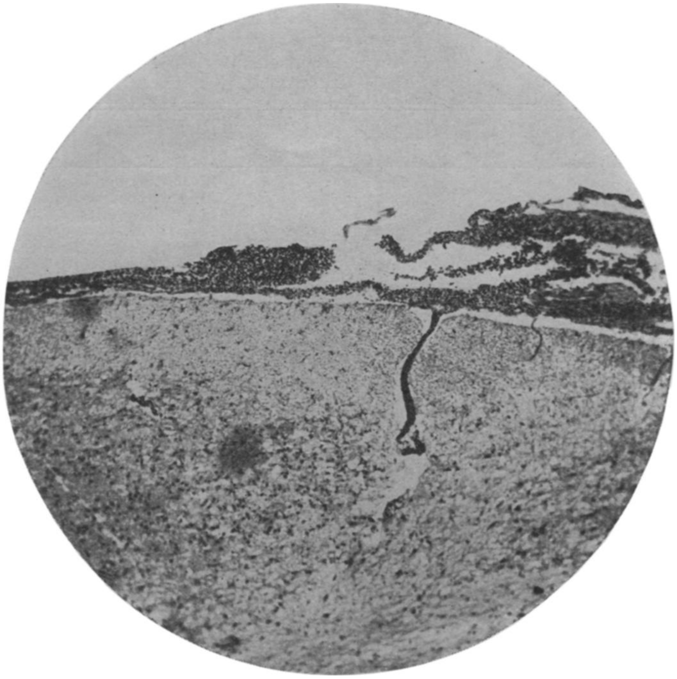

Fig. 4.—Section of spinal cord of rabbit in dorsal region removed seven days after the injection of the same culture in similar dosage employed in the infection of the meninges shown in figure 3, but followed four hours later by an injection of 0.5 c c of 1: 1,000 ethylhydrocuprein per kilogram of weight; shows slight leukocytic infiltration of the meninges. This animal was rapidly recovering when killed for microscopic study; × 70.

Exposure of virulent pneumococci to solutions of sodium oleate appeared to increase their susceptibility to the pneumococcidal activity of ethylhydrocuprein; the experiment shown in table 13 was conducted by cultivating the type 2 strain in glucose blood broth for 24 hours and dividing it into two parts of 4 c c each; to one part was added 0.5 c c of a 1: 1,000 solution of Merck's sodium oleate and to the second part 0.5 c c of salt solution as a control. Both mixtures were placed in an incubator for an hour followed by thorough centrifuging and resuspension of the cocci in 2.5 c c of warm physiologic salt solution. Both emulsions were then injected intraperitoneally in mice in equal doses followed in an hour by the intraperitoneal injection of varying doses of ethylhydrocuprein. The controls receiving the soaped and plain or untreated diplococci died in about 12 hours; mice receiving ethylhydrocuprein lived for varying periods up to 6 days and longer, and while the results were not regular, a larger percentage of mice infected with soaped pneumococci survived than of those infected with untreated cultures, indicating the protective value of ethylhydrocuprein especially against pneumococci exposed to sodium oleate.

TABLE 13

THE INFLUENCE OF ETHYLHYDROCUPREIN HYDROCHLORID ON SOAPED AND PLAIN PNEUMOCOCCI (TYPE 2)

Mouse Weight	Culture 0.5 c c	Ethylhydrocuprein	Results
18	Soaped	0.5 c c of 1:500	Lived over six days
22	Soaped	0.5 c c of 1:1.000	Lived over six days
20	Plain	0.5 c c of 1:500	Lived three days
21	Plain	0.5 c c of 1:1.000	Lived over six days
20	Soaped	None	Died in 12 hours (culture control)
20	Plain	None	Died in 12 hours (culture control)

In pneumococcus meningitis in rabbits the results were even more irregular than with mice, as the lesions were more involved; subthecal injection of mixtures of ethylhydrocuprein and sodium oleate appeared to prolong the lives of some infected animals and not of others as compared with animals receiving ethylhydrocuprein alone. These results are expressed quite well in the experiment shown in table 14 in rabbits with type 2 meningitis treated by the subthecal injection of various mixtures prepared as follows:

Mixture A:	Ethylhydrocuprein 1: 1,000	
Mixture B:	Ethylhydrocuprein 1: 100	1 c c
	Sodium oleate (Merck), 0.5%	1 c c
	Sodium solution	8 c c
Mixture C:	Ethylhydrocuprein 1: 100	1 c c
	Boric acid (5%)	2 c c
	Salt solution	7 c c
Mixture D:	Ethylhydrocuprein 1: 100	1 c c
	Sodium oleate (0.5%)	1 c c
	Boric acid (5%)	1 c c
	Salt solution	7 c c
Mixture E:	Sodium oleate (0.5%)	5 c c
	Boric acid (5%)	5 c c

Mixtures of ethylhydrocuprein and sodium oleate in salt solution were turbid; mixtures of ethylhydrocuprein and boric acid were quite clear.

Rabbits were infected by the subthecal injection of 0.01 c c of 24-hour broth cultures of virulent type 2 pneumococci, followed 2 hours later by the subthecal injection of 1 c c of each of the above mixtures.

As shown in this particular experiment, the control died in 48 hours with suppurative leptomeningitis and the animal receiving ethylhydrocuprein alone (A) survived longer than the animal receiving a mixture of ethylhydrocuprein and sodium oleate. Curiously, the animal receiving a mixture of ethylhydrocuprein and boric acid (C) survived indefinitely as did the animal receiving a mixture of sodium oleate and boric acid (E).

TABLE 14

INFLUENCE OF SODIUM OLEATE, BORIC ACID AND ETHYLHYDROCUPREIN HYDROCHLORID ON TYPE 2 MENINGITIS OF RABBITS

Weight in Grams	Drug†	Results
1,700	1 c c mixture A	Lived 9 days
2,000	1 c c mixture B	Lived 3 days
2,000	1 c c mixture C	Lived indefinitely
1,500	1 c c mixture D	Lived 5 days
2,000	1 c c mixture E	Lived indefinitely (control)
1,800	Cultured control	Died in 48 hours

* Rabbits infected by intraspinal injection of 0.01 c c of a 24-hour broth culture of type 2 (fatal for mice of 20 gm. in a dose of 0.0001 c c in 48 hours).

† Injected subthecally 2 hours after injection.

While sodium oleate undoubtedly reduces the vitality of pneumococci or at least increases their susceptibility to the destructive influences of ethylhydrocuprein when the organisms are exposed to the oleate in vitro, our results in the treatment of experimental pneumococcus meningitis in rabbits with mixtures of ethylhydrocuprein and sodium oleate have not yielded the encouraging results observed by Lamar in the treatment of experimental meningitis in monkeys with mixtures of specific antipneumococcus serum, boric acid and sodium oleate.

THE INFLUENCE OF MERCUROPHEN ON EXPERIMENTAL PNEUMOCOCCUS MENINGITIS IN RABBITS

As previously stated, the irritant properties of sodium oxymercury ortho-nitro-phenolate (Mercurophen) on the meninges following subthecal injection in rabbits, has not permitted the injection of a single dose sufficiently large to raise the germicidal activity of the spinal fluid to such a degree as will influence the course of a pneumococcus meningitis. Assuming that a 2,000 gm. rabbit has at least 2 c c of cerebrospinal fluid, the subthecal injection of 0.5 c c of a 1:2,000 solution of mercurophen which is the largest dose producing no irritation of the meninges, results in a final dilution of about 1:8,000 mercurophen which is too high for marked pneumococcidal activity on the basis of the results of germicidal tests in vitro with purulent spinal fluid, showing that mercurophen in dilutions higher than 1:4,000 exerts slight or no germicidal effect on pneumococci (table 5) under these conditions.

Experiments consisting in the infection of rabbits by subthecal injection of the type 2 pneumococcus followed 1, 4 and 24 hours later by the subthecal injection of solutions of mercurophen as high as 1:1,000 have had no appreciable effect on the course of the meningitis, the treated animals dying with leptomeningitis and showing pneumococci in the spinal fluid and blood of the heart. These results agree with those reported by Kolmer and Steinfield[10] showing that the intravenous and intramuscular injections of single doses of various mercurials including mercurophen, alone and in combination with pneumococcus serum, had no appreciable effect on prolonging the lives of mice infected by the intraperitoneal injections of type 1 pneumococci lethal in 72 hours; with the repeated subthecal injection of smaller doses of mercurophen in the treatment of a milder form of experimental meningitis as that produced by our type 1 strain of pneumococcus, some effect on the course of the meningitis was usually apparent, as best shown by the prolongation of the lives of the treated animals (table 15).

TABLE 15

THE INFLUENCE OF MERCUROPHEN ON TYPE 1 PNEUMOCOCCUS MENINGITIS

Weight in Grams	Culture*	Mercurophen		Results
		Dose	Injected	
1,200	0.01 c c	0.5 c c of 1:5,000	1st and 3rd days	Lived 8 days; acute meningitis
1,200	0.01 c c	None	....	Lived 4 days; acute meningitis
1,000	None	0.5 c c of 1:5,000	1st and 3rd days	Developed paralysis after second injection; recovered

* Minimum lethal dose for mice 0.001 c c in 48 hours; rabbits injected intraspinally on first, second and fourth days.

DISCUSSION

Our experiments have been severe tests for the protective and curative properties of the drugs included in this study, because it was necessary to infect the subarachnoid space with relatively large doses of virulent pneumococci in order to produce a definite train of lesions and symptoms, and owing to the marked susceptibility of the rabbits to invasion by the pneumococcus, the experimental infection was usually severe and rapidly fatal. The effects following the administration of the medicaments were judged mainly on the basis of duration of life of the treated animals as compared with the untreated controls; owing to the difficulty of securing spinal fluid from rabbits during life repeated chemical, cytologic and bacteriologic examinations of the fluid could not be made which would have yielded more evidence of a valuable character on the progress of the infection and effect of treatment. Histologic examinations of the brain, cord and meninges were made of every animal, but we have found these changes too irregular at different levels and areas to permit comparative studies and deductions.

Owing to the highly fatal nature of pneumococcus meningitis, the subject is particularly worthy of investigation, and we believe that ethylhydrocuprein has given some encouragement, providing the drug is injected subthecally early in the disease. According to the results of toxicity tests, it would appear safe to inject persons subthecally with 0.5 c c of a 1: 1,000 dilution per kilo of weight once or twice in 24 hours for a period of several days following the withdrawal of spinal fluid; this treatment in conjunction with appropriate surgical measures to effect local drainage in those cases of meningitis following a primary infection of the mastoid cells, frontal sinus or other accessible areas, is likely to modify the severity of the infection and may result in lowering the mortality of the disease.

The dose of ethylhydrocuprein advised would amount to 30 c c of a 1: 1,000 solution for a young adult weighing 60 kilo or about 125 pounds; this is equivalent to 0.0005 gm. of the drug per kilo of weight, and being at least 60 times less than the toxic dose, may be accepted as safe even when given once or twice a day for 4 or 5 days. With the withdrawal of spinal fluid and the injection of this amount of drug, a distinct pneumococcidal activity is imparted to the spinal fluid, the duration depending on absorption and requiring further study.

SUMMARY

Both ethylhydrocuprein and mercurophen are slightly more toxic by subthecal injections in the lumbar regions of normal rabbits than by intravenous injection.

Ethylhydrocuprein hydrochlorid in a dose of 0.03 to 0.05 gm. dissolved in 0.5 c c saline solution per kilo of body weight of rabbits produces leptomeningitis especially well defined at the site of injection, and is lethal in about 48 hours; in a dose of 0.5 c c of a 1: 1,000 solution per kilo of body weight the drug produces no irritation of the meninges, and is well borne by normal rabbits.

Mercurophen in a dose of 0.002 to 0.004 gm. in 0.5 c c salt solution per kilo of body weight, produces severe hemorrhagic leptomeningitis in rabbits and is lethal in about 58 hours; 1 c c of a 1: 2,000 dilution per kilo of weight injected subthecally produces slight or no irritation of the meninges, and is well borne for an indefinite period of time.

Germicidal tests in vitro with purulent spinal fluids containing virulent type 2 and type 1 pneumococci, have shown ethylhydrocuprein and mercurophen pneumococcidal in final dilution of 1: 4,000 in an exposure of one hour at 37 C.; the latter acts somewhat more rapidly than the former.

The subthecal injection of single doses of ethylhydrocuprein in amounts of 0.5 c c, of 1: 500 and 1: 1,000 solutions per kilo of body weight, had a distinct beneficial effect on the course of experimental meningitis in rabbits produced by a type 1 pneumococcus of moderate virulence, when administered not later than 4 to 6 hours after the injection of organisms; when given 24 hours after the pneumococci this effect was not apparent. With a meningitis produced by a more

virulent culture of type 2 pneumococci, single doses of this drug had but slight or no effect on the duration of the lives of the experimental animals.

In experiments consisting in the repeated subthecal injection of smaller doses of pneumococci followed in a few hours by ethylhydrocuprein, the protective and curative effects of the drug were more apparent.

Mixtures of specific antipneumococcus serum and ethylhydrocuprein did not yield results in the treatment of experimental meningitis produced by type 1 pneumococci, superior to those observed with the drug alone; the serum alone was without appreciable effect on the course of the experimental lesions.

Exposure of virulent pneumococci to solutions of sodium oleate in vitro increased their susceptibility to the germicidal activity of ethylhydrocuprein, but the subthecal injection of mixtures of sodium oleate and ethylhydrocuprein did not yield results in the treatment of experimental pneumococcus meningitis superior to those observed with ethylhydrocuprein.

The subthecal injection of single doses of mercurophen in amounts as large as 0.5 c c of 1: 1,000 solution per kilo of body weight was without appreciable effect on the course of experimental meningitis.

The repeated injection of smaller doses of type 1 pneumococci of moderate virulence followed in a few hours by the subthecal injection of smaller doses of mercurophen, resulted in prolonging the lives of some of the experimental animals.

In the treatment of pneumococcus meningitis of persons it is recommended that spinal puncture be made once or twice every day during the acme of the infection with the withdrawal of spinal fluid followed by the injection of a sterile and warm solution of ethylhydrocuprein hydrochlorid in physiologic salt solution in a dose of 0.5 c c of 1: 1,000 per kilo of body weight; in cases of meningitis following infection of the mastoid cells and accessory nasal sinuses, local drainage should be instituted by surgical measures.